

CLAIMS

1. A method of identifying the presence of a target nucleic acid in a sample wherein the target nucleic acid is replicated and said replication of the target is detected as the consumption of a deoxynucleotide triphosphate precursor, the method comprising:

5 adding an oligonucleotide primer which hybridizes to the sample target nucleic acid to the sample;

10 subjecting the sample DNA and primer to a polymerase reaction in the presence of a mixture of all dNTP's required for replication of the target nucleic acid whereby the deoxynucleotides will become incorporated and release pyrophosphate (PPi) proportional to the length of the DNA extension product; and

15 detecting any release of PPi enzymatically;

whereby any release of PPi is indicative of incorporation of deoxynucleotide or dideoxynucleotide and the presence of the target DNA.

2. A method as in claim 1, wherein the target nucleic acid is replicated in a reaction selected from the group consisting of a polymerase extension reaction, a polymerase chain reaction(PCR), a ligase chain reaction (LCR), a rolling circle replication reaction (RCR) and a nucleic acid sequence based amplification reaction (NASBA).

25 3. A method as in claim 2 wherein the target is replicated in a polymerase chain reaction.

4. A method as claimed in claim 1, wherein the release of PPi is detected by means of a Luciferase-Luciferin-based reaction.

30 5. A method as claimed in claim 1, wherein PPi release is detected using ATP sulfurylase and luciferase.

6. A method as claimed in claim 1, wherein the PPi detection enzymes are

included in the polymerase reaction step and the polymerase reaction and PPi release detection steps are performed substantially simultaneously.

7. A method as claimed in claim 1, further comprising adding a dATP analogue
5 which is capable of acting as a substrate for a polymerase, but incapable of acting as a substrate for a PPi detection enzyme.

8. A method as claimed in claim 7, wherein the dATP analogue is deoxyadenosine .alpha. thiotriphosphate

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9. A method as claimed in claim 1, wherein the sample DNA or oligonucleotide primer is immobilized or provided with means for attachment to a solid support.

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10. A method as claimed in claim 1, wherein an exonuclease deficient high fidelity polymerase is used.

11. A method as in claim 1 wherein a heat resistant polymerase is used.

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12. A method as claimed in claim 1, wherein the sample DNA is first amplified.

13. A method as claimed in claim 1, for use with a multiplicity of sample DNA sequences, wherein said DNA sequences are arranged in assay format on a solid surface.

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14. The method according to claim 1 wherein said nucleic acid sample is obtained from a biological sample.

15. The method according to claim 1 wherein said target nucleic acid is a microbial or viral nucleic acid.

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16. The method according to claim 15 wherein said target nucleic acid target is a viral nucleic acid.

17. The method according to claim 1 wherein said nucleic acid sample is obtained from a food source.

5 18. The method according to claim 17 wherein said food source is a plant.

19. The method according to claim 1 wherein said target nucleic acid contains a nucleic acid sequence non-native to the sample.

10 20. A kit for use in a method as defined in claim 1, comprising:
a polymerase;
detection enzyme means for identifying PPi release;
dNTP's, or optionally deoxynucleotide analogues, optionally including, in place of dATP, a dATP analogue which is capable of acting as a substrate for a
15 polymerase but incapable of acting as a substrate for a said PPi-detection enzyme; and
optionally a target specific primer which hybridizes to the target DNA and is recognized as a primer by a polymerase, wherein the polymerase replicates the target DNA.

20 21. A kit as claimed in claim 20, wherein the detection enzyme means comprise a Luciferase-Luciferin-based enzymatic reaction.

22. A kit as claimed in claim 21, wherein the detection enzyme means comprise ATP sulfurylase and luciferase.

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